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The internal surface area of the adult human lung

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INTRODUCTION

The internal surface area of the lung comprises alveoli, alveolar ducts and the alveoli arising from respiratory bronchioles. It thus equals the air/tissue interface available for gas exchange. Measurement of the internal surface area of the normal lung allows correlation between physiological studies of lung function and morphological studies of the normal and diseased lung. Normal adult lungs from only eight cases have been studied (Dunnill, 1962*b*; Weibel, 1963; Dunnill, 1964; Heath, Brewer & Hicken, 1968) using the method of formalin steam fixation described by Weibel & Vidone (1961). This technique has the advantages that the fixed lung does not collapse on sectioning and there is uniform tissue shrinkage (Weibel, 1968). In the present study a series of 28 normal lungs were fixed in inflation by formalin steam and their internal surface area was determined. The internal surface area was then related to age, height and body weight. Regression equations for the internal surface area, and other quantitative pulmonary factors to be described below, on age, height and body weight were also determined. Such equations are useful in establishing a baseline for comparison with cases of pulmonary disease (Hasleton, 1971).

MATERIALS AND METHODS

Cases studied

Twenty-eight subjects with normal lungs were studied. Nine were males and nineteen females. The subject's number, age, sex and cause of death are shown in Table 1. Any subject with mitral or aortic valve disease, congenital heart disease, chronic left ventricular failure, pulmonary emphysema, interstitial pulmonary fibrosis or bronchiectasis was not included in the series. The right lung was studied in three cases (subjects 15, 22 and 26) and the left lung was studied in the remainder.

Fixation and macroscopic point-counting of the lungs

The lungs were removed from the chest with meticulous care. Both main bronchi were divided at the carina and any mucus in the bronchial tree was aspirated. The lung was fixed in inflation by a modification of the method of Weibel & Vidone (1961) as described by Hicken, Heath & Brewer (1966*a*). The volume shrinkage factor and the volume of the fixed lung were found after the amount of shrinkage occurring during fixation had been determined (Hicken *et al.* 1966*a*). The fixed lung was cut into sagittal slices 1 cm thick.

The lung slices were point-counted to determine the percentage of bronchovascular and parenchymatous tissue (Dunnill, 1962*a*; Weibel, 1963). Bronchovascular tissue was defined as pleural surface, bronchi and pulmonary blood vessels greater than 0·2 cm in diameter. This tissue plays no part in the gas exchange which occurs in the lung and the percentage was determined for subsequent calculations.

Quantitative histological studies

Randomly sampled blocks of lung were taken for histology. Sections, 6 μm thick, were cut and stained with the Lawson modification of the Weigert-Sheridan method for elastin and a Van Gieson counterstain for fibrous tissue and collagen. The amount of shrinkage occurring during processing was determined (Hicken, Brewer & Heath, 1966*b*).

The internal surface area was measured by the method of the mean linear intercept as described by Weibel & Gomez (1962) and Dunnill (1962*a*). The mean linear intercept (M.L.I.) was derived from the following formula:

$$L_m = \frac{NT}{I}, \tag{1}$$

where L_m = mean linear intercept, N = number of fields studied on each slide, T = mean length of the traversing lines and I = total number of intercepts.

The internal surface area (I.S.A.) per unit volume can be found from equation (2) below:

$$S_p/\text{unit volume} = \frac{4}{L_m}, \tag{2}$$

where S_p = internal surface area of the processed lung, L_m = mean linear intercept. The I.S.A. of the fresh lung is determined from the following formula:

$$S_F = \frac{4}{L_m} \times V_p \times \alpha \times f^2 \times p^2, \tag{3}$$

where S_F = internal surface area of the fresh lung, L_m = mean linear intercept, V_p = volume of the processed lung and α = fraction of lung occupied by parenchyma (i.e. 100 − % bronchovascular tissue and expressed as a fraction): f^2 = area fixation factor from fresh to fixed tissue, p^2 = area processing factor from fixed to processed tissue.

Internal surface area at a standard lung volume

It can be seen from equation (3) above that the lung volume is an important variable in determining the I.S.A. To try to compensate for this factor the I.S.A. at a standard lung volume of 3000 ml (I.S.A.$_{3000}$) was found using an equation given by Duguid, Young, Cauna & Lambert (1964).

$$S_{3000} = \left(\frac{3000}{V_f}\right)^{\frac{2}{3}} \times S_F, \tag{4}$$

where S_{3000} = internal surface area at a standard lung volume of 3000 ml, V_f = volume of the fresh lung and S_F = internal surface area of the fresh lung.

The M.L.I., I.S.A. and I.S.A.$_{3000}$ were correlated with age, height and body weight. From these correlations it was possible to derive regression equations giving predictions of M.L.I., I.S.A. and I.S.A.$_{3000}$ for a known age, height or body weight.

Table 1. *Sex, age and diagnosis at necropsy*

Case no.	Sex	Age (years)	Diagnosis at necropsy
1	M	44	Subarachnoid haemorrhage
2	M	53	Bronchopneumonia. Perforated appendix
3	M	59	Coronary thrombosis. Diabetes mellitus
4	M	28	Proliferative glomerulonephritis
5	M	39	Pulmonary embolism. Gastric ulcer
6	M	58	Carcinoma of stomach
7	M	55	Proliferative glomerulonephritis
8	M	65	Coronary thrombosis. Carcinoma of stomach
9	M	50	Staphylococcal septicaemia. Maxillary sinusitis
10	F	65	Peritonitis. Reticulosarcoma of jejunum
11	F	65	'Malignant nasal granuloma'
12	F	54	Cerebral abscess. Chronic mastoiditis
13	F	59	Subarachnoid haemorrhage
14	F	44	Pancytopenia
15	F	55	Astrocytoma
16	F	77	Cerebellar softening
17	F	71	Ruptured abdominal aortic aneurysm
18	F	66	Chemodectoma of lung. Cardiac arrest
19	F	61	Postcricoid carcinoma
20	F	49	Cerebral haemorrhage
21	F	50	Carcinoma of thyroid
22	F	60	Primary carcinomata of ovary and colon
23	F	82	Renal abscess. Recurrent papillomata of bladder
24	F	82	Ruptured pseudomucinous cystadenoma of ovary
25	F	31	Chronic pyelonephritis
26	F	68	Cerebral embolism. Myocardial infarction
27	F	72	Pulmonary embolism. Abdominal aortic aneurysm
28	F	64	Acute pancreatitis

RESULTS

The ages of the subjects studied ranged from 28 to 82 years (Table 1). The volumes of the fresh and fixed lungs and the linear fixation and processing factors are shown in Table 2. The volume of the fresh lung ranged from 2157 to 5225 ml, and that of the fixed lung from 1360 to 4010 ml. The value of f ranged from 0·98 to 1·52. Case 7 was the only case with a value of f below 1. This was because the lung was oedematous and not fully inflated at the start of fixation, so that, as formaldehyde vapour penetrated the lung substance, the volume increased. Only two cases had values of f greater than 1·2. The values of p were in a narrower range than those of f, the range being 1·07–1·34. Eighteen of the cases had values between 1·2 and 1·3.

The height, body weight, M.L.I., I.S.A. and I.S.A._{3000} are given in Table 3. The heights ranged from 147 to 188 cm, the range of the weights being 26–83 kg.

The mean linear intercept had a range of $190{\cdot}21–276{\cdot}38 \times 10^{-4}$ cm. Only two cases (cases 17 and 20) had a M.L.I. greater than 260×10^{-4} cm. The I.S.A. ranged from 23·56 to 68·76 m^2. Two cases (cases 1 and 27) had values greater than 50 m^2 and 11 had values greater than 40 m^2. The corresponding figures for the I.S.A._{3000} were lower than for the I.S.A. and ranged from 28·93 to 47·57 m^2. Height was significantly correlated with both age and body weight ($r = -0{\cdot}492$, $t = 2{\cdot}879$, $P < 0{\cdot}01$: $r = +0{\cdot}580$, $t = 3{\cdot}629$, $P < 0{\cdot}005$ respectively), the correlation between height and age

Table 2. *Volumes of fresh and fixed lungs and values of f and p*

Case no.	Volume of fresh lung (ml)	Volume of fixed lung (ml)	f	p
1	5225	4010	1·09	1·21
2	3781	3740	1·003	1·32
3	3908	2490	1·16	1·22
4	2241	1360	1·18	1·24
5	4020	3480	1·05	1·23
6	3514	3110	1·04	1·21
7	2157	2210	0·98	1·22
8	2938	2780	1·02	1·23
9	3424	3410	1·001	1·24
10	2217	1955	1·04	1·21
11	3350	2100	1·17	1·21
12	5000	1410	1·52	1·21
13	3962	2850	1·12	1·19
14	2680	2560	1·02	1·34
15	3573	3170	1·04	1·26
16	2205	1760	1·07	1·19
17	2824	2215	1·08	1·07
18	3653	3115	1·05	1·28
19	2787	2550	1·03	1·22
20	3462	2790	1·07	1·18
21	3809	2890	1·20	1·19
22	4385	3340	1·09	1·21
23	3398	2580	1·10	1·21
24	3534	2810	1·08	1·23
25	2504	1920	1·09	1·29
26	2430	2058	1·06	1·17
27	4738	1650	1·42	1·16
28	2374	2175	1·03	1·18

being negative. Age and height both showed significant correlations with M.L.I., that between M.L.I. and height being negative (Fig. 1), and that between age and M.L.I. being positive (Fig. 2). The only other significant correlations were between height and I.S.A. (Fig. 3) and height and I.S.A.$_{3000}$ (Fig. 4).

As two factors, age and height, play an important role in determining the value of the M.L.I., and as the I.S.A. and the I.S.A.$_{3000}$ are derived from the M.L.I., simple correlation coefficients between two variables such as M.L.I. and height are not adequate. Thus multiple correlation coefficients (R) and F tests were carried out on the M.L.I., I.S.A. and I.S.A.$_{3000}$ respectively on the one hand and age, height and body weight on the other. The F test can be compared to the 't' test, but there are more than two variables. To determine the effect of age, height and body weight on the final values of the M.L.I., I.S.A. and I.S.A.$_{3000}$, regression equations were calculated and values of t determined when each true gradient in turn was assumed to be equal to 0.

The values of R, F and P, the regression coefficients, and their values of t and P are shown in Table 4. Only one F test was significant, that between the M.L.I. and age, height and weight. It can be seen from the regression coefficients that height contributed most to the M.L.I. Though the regression coefficient for age and M.L.I. is not significant, the value of t is near to that needed for a significant value (P = 0·05

Table 3. *Height, weight, mean linear intercept, internal surface area and internal surface area of a standard lung volume in the cases studied*

Case no.	Height (to nearest cm)	Weight (to nearest kg)	M.L.I. (10^{-4} cm)	I.S.A. (m^2)	I.S.A.$_{3000}$ (m^2)
1	188	71	190·21	68·76	47·57
2	175	51	201·79	46·90	40·19
3	170	70	245·45	37·63	31·92
4	188	59	203·69	25·75	31·29
5	183	60	223·03	48·89	40·31
6	173	67	236·50	41·39	37·25
7	168	67	218·51	27·16	33·87
8	175	49	218·53	35·51	36·01
9	175	52	195·71	49·48	45·31
10	170	48	222·87	26·78	32·78
11	170	49	210·66	38·01	35·34
12	157	56	209·88	45·76	32·56
13	173	60	225·39	46·62	38·73
14	170	82	205·59	33·13	35·68
15	168	57	209·93	49·70	44·24
16	152	26	244·93	23·56	28·93
17	162	56	270·83	28·69	29·87
18	170	44	221·79	41·54	36·42
19	157	32	226·47	34·08	35·78
20	155	35	276·38	32·74	31·74
21	175	76	251·04	39·22	33·44
22	170	70	249·84	47·23	36·66
23	152	36	250·77	35·92	33·09
24	175	83	245·51	36·65	32·86
25	168	73	200·88	30·11	33·96
26	147	45	250·49	26·21	30·16
27	160	56	225·74	59·87	44·14
28	160	52	244·87	27·47	32·11

when $t = 2{\cdot}063$). Similarly, height contributed most to the I.S.A., though neither the F test nor the regression coefficient was significant. The regression coefficient for height and I.S.A.$_{3000}$ was significant. Thus the regression equations for I.S.A. and I.S.A.$_{3000}$ could be obtained using height only. These equations are given below:

$$\text{I.S.A.} = -32{\cdot}962 + 0{\cdot}426 \times \text{height (in cm)}, \qquad (5)$$

$$\text{I.S.A.}_{3000} = 0{\cdot}239 + 0{\cdot}211 \times \text{height (in cm)}. \qquad (6)$$

The regression equation for M.L.I. took both height and age into account and was adjusted to exclude weight.

$$\text{M.L.I.} = 227{\cdot}767 + 0{\cdot}601(\text{age in years} - 58{\cdot}786) - 0{\cdot}822(\text{height in cm} - 168{\cdot}161), \qquad (7)$$

where $227{\cdot}767$ = mean M.L.I., $0{\cdot}601$ = regression gradient of M.L.I. on age when regression gradient of M.L.I. on height = 0, $58{\cdot}786$ = mean age, $0{\cdot}822$ = regression gradient of M.L.I. on height when regression gradient of M.L.I. on age = 0, $168{\cdot}161$ = mean height.

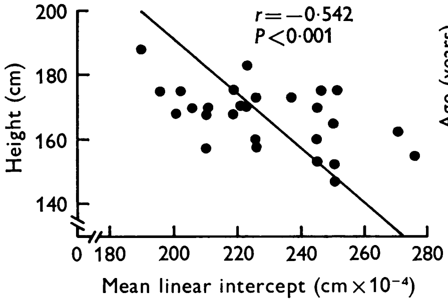

Fig. 1

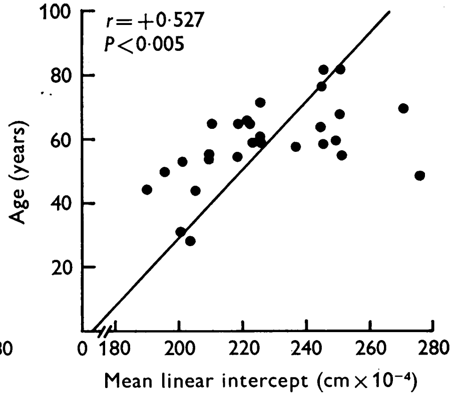

Fig. 2

Fig. 1. Relation between height and mean linear intercept in subjects with normal lungs. The calculated regression line is shown.

Fig. 2. Relation between age and mean linear intercept. The line is the calculated regression line.

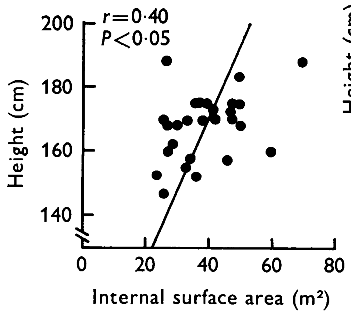

Fig. 3

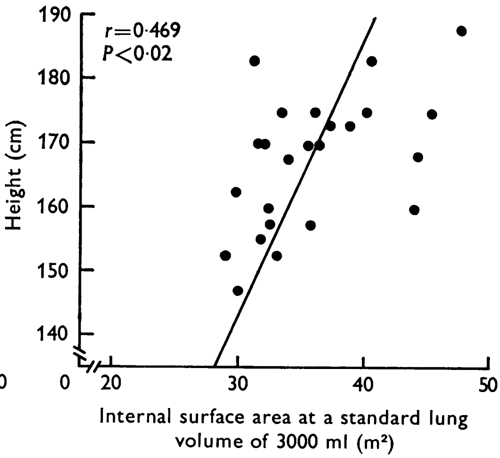

Fig. 4

Fig. 3. Relation between the height and internal surface area. Calculated regression line shown.

Fig. 4. Relation between height and the internal surface area at a standard lung volume of 3000 ml. Calculated regression line shown.

DISCUSSION

Previous quantitative histological studies on the acini of the lung have concentrated on the I.S.A. in normal and emphysematous lungs (Dunnill, 1962*b*, 1964, 1965; Weibel, 1963; Duguid *et al.* 1964; Thurlbeck, 1967*a*, *b*; Heath *et al.* 1968). The I.S.A. is not an accurate measurement of the air/tissue interface since it is affected by seven variables (equation 1). On the other hand the equation for the determination of the M.L.I. has only three variables (equation 3). Two of these variables, the number of

Table 4. *Values of R, F and regression coefficients when* M.L.I., I.S.A. *and* I.S.A.$_{3000}$ *are respectively compared with age, height and body weight*

Correlation between		*R*	*F*	*P*	Regression coefficient		*t*	*P*
M.L.I. and	age				0·6393	(age)	2·02	<0·1
	height	0·6393	5·5303	<0·01	−1·0461	(height)	−2·29	<0·05
	weight				0·2991	(weight)	1·00	<0·4
I.S.A. and	age				0·1285	(age)	0·71	<0·5
	height	0·4218	1·7310	>0·05	0·4737	(height)	1·82	<0·1
	weight				0·003944	(weight)	0·23	<0·9
I.S.A.$_{3000}$ and	age				0·00946	(age)	0·12	<0·95
	height	0·4798	2·3926	>0·05	0·2642	(height)	2·33	<0·05
	weight				−0·00397	(weight)	−0·53	<0·6

Table 5. *The* M.L.I., I.S.A. *and* I.S.A.$_{3000}$ *in normal lungs in the present and other series*

Author(s)	M.L.I. (10^{-4} cm)	I.S.A. (m^2)	I.S.A.$_{3000}$ (m^2)
Dunnill (1962*b*, 1964)	208·5, 286·79	37·5, 37·9	22·75, 25·03
Weibel (1963)	188·0 –249·0	36·9 –40·6	23·37–26·31
Duguid *et al.* (1964)	—	28·0 –42·0	38·0 –44·0
Thurlbeck (1967*a*)	172·0 –282·0	20·0 –48·5	16·42–25·83
Heath *et al.* (1968)	—	23·95–41·4	19·36–26·31
Present series	190·21–276·38	23·56–68·76	28·93–47·57

fields studied and the mean length of the traversing lines, are constant values in most cases. Thus the M.L.I. is a much more reliable indication of permanent enlargement of alveoli or alveolar ducts, such as occurs in emphysema. It must be noted that the M.L.I. does not refer to any alveolar or acinar dimension. In cases with normal lungs the M.L.I. has a much narrower range, $190{\cdot}21–276{\cdot}38 \times 10^{-4}$ cm, than the I.S.A.

The largest factor in the equation for the determination of the I.S.A. is the volume of the processed lung. It is difficult to know the true volume of the fresh, and thus of the processed lung at post-mortem, since the volume of the fresh lung depends on the inflating pressure, and it is impossible to know the correct pressure for each case. There is further evidence that present methods of fixation do not show the true I.S.A. When greyhounds are frozen in a vertical (head uppermost) position there is a progressive decrease in alveolar size from the apex to the base of the lung (Glazier, Hughes, Maloney, Pain & West, 1966). When human lungs removed at necropsy but fixed with formaldehyde vapour are studied in a similar manner there is no difference between the alveolar size at the apex or base of the lung (Cumming, 1968).

To compensate for the errors inherent in the method of measuring the I.S.A., the I.S.A. at a standard lung volume was used. This is a more useful guide for comparison of different cases, since the standard deviation of the predicted I.S.A.$_{3000}$ (4·56) is lower than the corresponding value for the predicted I.S.A. (10·47) (Hasleton, 1971).

The most accurate guide to the enlargement of alveoli and alveolar ducts was the

M.L.I. as it was not affected by the lung volume. Whereas the I.S.A. and I.S.A.$_{3000}$ were only related to height, the M.L.I. was related to both height and age. Other authors' values for the M.L.I., I.S.A. and I.S.A.$_{3000}$ are given in Table 5. Two of Weibel's subjects and two subjects quoted in the monograph by Heath *et al.* (1968) have been omitted from the table as they were below 20 years of age. When the I.S.A. for both lungs is given by the authors, the figure in the table is their value divided by two. All the authors except Thurlbeck (1967*a*) fixed the lungs with formaldehyde vapour.

The values of the M.L.I. compare satisfactorily in the three series in which this parameter is available. Similarly there is close agreement between the I.S.A. in the present and other series since the majority of cases in the present series had an I.S.A. below 40 m^2 (Table 3).

This agreement does not hold true when the I.S.A.$_{3000}$ obtained by other authors, except by Duguid *et al.* (1964), is compared with the present series. Only eight subjects fixed by formaldehyde vapour by the method of Weibel & Vidone have been studied and thus it is difficult to make a true comparison.

Thurlbeck (1967*a*) studied 25 subjects, examining both lungs, and relating the I.S.A. and M.L.I. to body length, body weight and age. The lungs were distended with intrabronchial 4 % formaldehyde. This method of fixation has been criticized (Weibel, 1968) since distortion of the alveolar spaces occurs around a 'core' of formaldehyde which has a constant volume. Using formaldehyde vapour the alveoli are not distorted as the vapour is compressible. Weibel also showed that with 4 % formaldehyde fixation there is unequal shrinkage of different parts of the tissue.

Thurlbeck took no account of shrinkage of lung tissue from the fresh to fixed lung volume in his calculation of the I.S.A. Only 'lateral slices' were examined and there is a theoretical risk that alveolar duct emphysema in the other slices may have been missed. Examination of one slice per lung is not statistically acceptable (Anderson & Dunnill, 1965).

For the above reasons an accurate comparison between Thurlbeck's results and those given in the present series is difficult. However, some interesting points emerge. The range of the M.L.I. is higher in material fixed with 4 % formaldehyde, seven of the subjects having an M.L.I. below 200×10^{-4} cm. These low values confirm Weibel's findings that aqueous formaldehyde causes unequal shrinkage of the alveolar spaces. The I.S.A. of the normal lung, as obtained by Thurlbeck, is significantly lower than in the lungs in the present series ($t = 2{\cdot}54$, $P < 0{\cdot}02$). This can be attributed to the smaller lung volumes obtained using aqueous formaldehyde. In contrast to the present series, that of Thurlbeck showed no significant correlation between age and height. This is not due to a difference in the age groups studied. The fact that as age increases height decreases is confirmed by previous studies, which have shown that with ageing there is a reduction in water content of the nucleus pulposus (Collins, 1949), an alteration in the protein–polysaccharide complex of the nucleus pulposus (Lyons, Jones, Quinn & Sprunt, 1964) and a progressive disc degeneration that occurs with ageing leading to kyphosis (Lawrence, 1969).

In the present series both height and age were related to the M.L.I. Thurlbeck, however, found no significant correlation between height and M.L.I. He did obtain a significant relation between age and the I.S.A. at a standard lung volume of 5000 ml. It was not possible to repeat this finding in the present series, in which the I.S.A. was standardized to 3000 ml. Most of the above differences may be explained by the differences in technique enumerated above.

This study is based on the assumption that there is no difference in the quantitative measurements in the right and left lungs. At the beginning of the investigation a case with minimal emphysema (1·2% in the left lung and 0·2% in the right) was studied. Such small amounts of emphysema were unlikely to make an appreciable difference to the mean linear intercept or the internal surface area. The quantitative measurements were normal in both lungs, and the differences in the M.L.I., I.S.A. and I.S.A.$_{3000}$ were respectively $13{\cdot}85 \times 10^{-4}$ cm, $6{\cdot}92$ m^2 and $6{\cdot}08$ m^2. The differences in these values were thought to be within the limits of experimental error.

SUMMARY

A series of 28 normal lungs was fixed in inflation by formaldehyde vapour. The percentages of normal and bronchovascular tissue were determined by macroscopic point-counting and the internal surface area (I.S.A.) determined by the method of the mean linear intercept (M.L.I.).

The M.L.I. ranged from $190\text{–}276 \times 10^{-4}$ cm. The corresponding values of the I.S.A. and I.S.A.$_{3000}$ (I.S.A. at a standard lung volume of 3000 ml) were 24–69 m^2 and 29–48 m^2. Most cases had an I.S.A. below 50 m^2 and an I.S.A.$_{3000}$ below 40 m^2.

The M.L.I. was the most accurate quantitative histological guide to the enlargement of air spaces in the lung. There was a significant negative correlation between height and M.L.I. and a significant positive correlation between age and M.L.I. Thus both age and height had to be taken into account in determining a regression equation for the M.L.I. There were significant positive correlations between height and both I.S.A. and I.S.A.$_{3000}$.

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